EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	870	nadeau.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 16:28
S2	382	S1 and @pd<"19990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 16:29
S3	11	S2 and hairpin	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:06
S4	494	tyagi.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:06
S5	164	S4 and @pd<"19990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 10:25
S6	2	S5 and beacon	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:19
S7	2581	molecular adj beacon	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:20
S8	14	S7 and @pd<"19990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:43
S9	1214	hairpin with probe	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:44
S10	0	S9 and @pd<"1990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:14
S11	0	hairpin and @pd<"1990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/08 17:46

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S12	10726	kramer.in.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 10:25
S13	6555	S12 and @pd<"19990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 10:26
S14	0	S13 and (chemokine adj receptor)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 10:27
S15	0	S13 and (wavelength adj shifting)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 13:35
S16	130	S13 and wavelength	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 10:28
S17	7	S16 and probe	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:13
S18	0	S17 and cacg	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:13
S19	0	S16 and CACG	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:13
S20	1214	hairpin with probe	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:14
S21	106	S20 and @pd<"19990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:15
S22	1	S21 and (CACG or GTGC or GCAC or CGTG)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 11:15
S23	905	wavelength adj shifting	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 13:35

EAST Search History

S24	247	S23 and @pd<"19990802"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 13:36
S25	0	S24 and (kramer.in. or tyagi.in.)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2006/08/09 13:36

BioMath Calculators

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Print Results

T_m Calculations for Oligos

Patent No: 6,037,130
SEQ ID NO: 11

There are a number of different ways to calculate the melting temperature (T_m) of an oligo. All of these methods will give different results. Please note that these calculations are theoretical. Optimum T_m values must be determined empirically.

For details on each of the calculations performed below please see the notes below, "Theoretical T_m of Oligos".

Step 1.

Enter your oligonucleotide sequence in the field below and press "Calculate." If you are using a Promega oligo, select from the drop-down menu below.
Also, if you know that the additional parameters of the reaction vary from the default values listed below, you may change these individually (in Step 2).

Note: Characters other than "G", "A", "T" and "C" will be ignored.)

CTTGTGGGTCAACCC

Calculate

or

select from the following Promega Primers

Optional Step 2.

Combined concentration of K^+ and Na^+ in the reaction: 50 mM
(**Note:** Promega's standard PCR* buffers contain 50mM K^+.)

Salt Concentration Adjustment: Please choose the product you are using or manually set the salt concentration above.

- (•) Set Manually
- ( ) Access RT-PCR System
- ( ) PCR Master Mix
- ( ) AccessQuick™ RT-PCR System.
- ( ) GoTaq™ Reaction Buffer

Note: Monovalent cation concentration will not affect the basic T_m calculations.

Primer concentration in the reaction: 270 nanomolar
Note: Primer concentration will only affect the base-stacking calculations.

[x] Adjust for Mg^{+2} concentration?
Mg^{+2} concentration in the reaction: 1.5 mM
Note: Promega's standard PCR buffers contain 1.5mM Mg^{+2}. Checking this box will only affect the base stacking calculations.

[ ] The oligo has a 5′-phosphate group
Note: Checking this box will change the calculated molecular weight but will not affect the temperature calculations.

RESULTS

Sequence analyzed: CTTGTGGGTCAACCC

The oligo is 60 % GC The oligo is 15 bases long

The molecular weight of the oligo is 4545 Daltons.

The basic T_m is 45 °C

The salt-adjusted T_m is 40 °C

The base-stacking calculated T_m is 57 °C

The base-stacking calculations were updated in September 2000. You may find that the predicted T_m values of known sequences will change. Please see the discussion below for details.

Thermodynamic Parameters:

ΔH -114 kcal/mol ΔS -315 cal/degree k mol

Theoretical T_m of Oligos

There are several formulas for calculating melting temperatures (T_m). In all cases these calculations will give you a good starting point for determining appropriate annealing temperatures for PCR, RT-PCR, hybridization and primer extension procedures. However, a **precise optimum annealing temperature must be determined empirically**.

Basic T_m Calculations

The simplest formula is as follows (1):

T_m = 4°C x (number of G's and C's in the primer) + 2°C x (number of A's and T's in the primer)

This formula is valid for oligos <14 bases and assumes that the reaction is carried out in the presence of 50mM monovalent cations. For longer oligos, the formula below is used:

T_m = 64.9°C + 41°C x (number of G's and C's in the primer – 16.4)/N

Where N is the length of the primer.

For example, Promega's T7 Promoter Primer (TAATACGACTCACTATAGGG) is a 20mer composed of 5 T's, 7 A's, 4 C's, and 4 G's. Thus, its melting temperature is calculated:

64.9°C + 41°C x (8 – 16.4)/20 = 47.7°C

Salt-Adjusted T_m Calculations

Another commonly used formula takes into account the salt concentration of the reaction (1–4). This formula has several variations, but all of them are essentially as follows:

T_m = 81.5°C + 16.6°C x ($\log_{10}[Na^+] + [K^+]$) + 0.41°C x (%GC) – 675/N

Where N is the number of nucleotides in the oligo. Note that PCR is typically performed in the presence of ~50mM monovalent cations.

Using the same T7 Promoter Primer as an example in PCR with 50mM monovalent cation concentration, its T_m is calculated:

81.5°C + 16.6°C x ($\log_{10}$[0.05]) + 0.41°C x (40) – 675/20 = 42.5°C

BioMath Calculators

T_m Calculations for Oligos

Whitcombe et al. (B2098)

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Print Results

There are a number of different ways to calculate the melting temperature (T_m) of an oligo. All of these methods will give different results. Please note that these calculations are theoretical. Optimum T_m values must be determined empirically.

For details on each of the calculations performed below please see the notes below, "Theoretical T_m of Oligos".

Step 1.

Enter your oligonucleotide sequence in the field below and press "Calculate." If you are using a Promega oligo, select from the drop-down menu below.
Also, if you know that the additional parameters of the reaction vary from the default values listed below, you may change these individually (in Step 2).

Note: Characters other than "G", "A", "T" and "C" will be ignored.)

ATGTAGCACATCAGAAG

Calculate

or

select from the following Promega Primers

Optional Step 2.

Combined concentration of K^+ and Na^+ in the reaction: 50 mM
(**Note:** Promega's standard PCR* buffers contain 50mM K^+.)

Salt Concentration Adjustment: Please choose the product you are using or manually set the salt concentration above.

- (•) Set Manually
- ( ) Access RT-PCR System
- ( ) PCR Master Mix
- ( ) AccessQuick™ RT-PCR System.
- ( ) GoTaq™ Reaction Buffer

Note: Monovalent cation concentration will not affect the basic T_m calculations.

Primer concentration in the reaction: 500 nanomolar
Note: Primer concentration will only affect the base-stacking calculations.

[x] Adjust for Mg^{+2} concentration?
Mg^{+2} concentration in the reaction: 1.5 mM
Note: Promega's standard PCR buffers contain 1.5mM Mg^{+2}. Checking this box will only affect the base stacking calculations.

[ ] The oligo has a 5´-phosphate group
Note: Checking this box will change the calculated molecular weight but will not affect the temperature calculations.

RESULTS

Sequence analyzed: ATGTAGCACATCAGAAG

The oligo is 41.18 % GC The oligo is 17 bases long

The molecular weight of the oligo is 5228 Daltons.

The basic T_m is 42 °C

The salt-adjusted T_m is 37 °C

The base-stacking calculated T_m is 55 °C

The base-stacking calculations were updated in September 2000. You may find that the predicted T_m values of known sequences will change. Please see the discussion below for details.

Thermodynamic Parameters:

ΔH -128 kcal/mol ΔS -361 cal/degree k mol

Theoretical T_m of Oligos

There are several formulas for calculating melting temperatures (T_m). In all cases these calculations will give you a good starting point for determining appropriate annealing temperatures for PCR, RT-PCR, hybridization and primer extension procedures. However, a **precise optimum annealing temperature must be determined empirically.**

Basic T_m Calculations

The simplest formula is as follows (1):

T_m = 4°C x (number of G's and C's in the primer) + 2°C x (number of A's and T's in the primer)

This formula is valid for oligos <14 bases and assumes that the reaction is carried out in the presence of 50mM monovalent cations. For longer oligos, the formula below is used:

T_m = 64.9°C + 41°C x (number of G's and C's in the primer – 16.4)/N

Where N is the length of the primer.

For example, Promega's T7 Promoter Primer (TAATACGACTCACTATAGGG) is a 20mer composed of 5 T's, 7 A's, 4 C's, and 4 G's. Thus, its melting temperature is calculated:

64.9°C + 41°C x (8 – 16.4)/20 = 47.7°C

Salt-Adjusted T_m Calculations

Another commonly used formula takes into account the salt concentration of the reaction (1–4). This formula has several variations, but all of them are essentially as follows:

T_m = 81.5°C + 16.6°C x ($\log_{10}[Na^+] + [K^+]$) + 0.41°C x (%GC) – 675/N

Where N is the number of nucleotides in the oligo. Note that PCR is typically performed in the presence of ~50mM monovalent cations.

Using the same T7 Promoter Primer as an example in PCR with 50mM monovalent cation concentration, its T_m is calculated:

81.5°C + 16.6°C x ($\log_{10}$[0.05]) + 0.41°C x (40) – 675/20 = 42.5°C

Base-Stacking T_m Calculations

The most sophisticated T_m calculations take into account the exact sequence and base stacking parameters, not just the base composition (1,5,6).

The equation used is:

$$T_m = \frac{\Delta H \ \frac{\text{kcal}}{\text{°C*Mol}}}{\Delta S + R \ln([\text{primer}]/2)} - 273.15°C$$

Where:

- ΔH is the enthalpy of base stacking interactions adjusted for helix initiation factors (6,7).
- ΔS is the entropy of base stacking adjusted for helix initiation factors (6,7) and for the contributions of salts[a] to the entropy of the system (6).
- R is the universal gas constant (1.987Cal/°C*Mol)

This equation, as implemented above, is valid if the following assumptions are met:

- The primer is not self complementary. For self-complementary oligos, the denominator of the equation becomes ΔS + R ln ([primer]/4)
- The primer concentration is much greater than the target concentration[b]. If the concentrations are almost equal, the denominator of the equation becomes ΔS + R ln([primer] - [target]/2)
- The primer is an "oligo" rather than a long polymer. The salt effects on polymers is significantly different from those on oligos. For a complete discussion, see reference 6.

For a complete discussion of the parameters involved in base-stacking calculations of T_m, see references 5, 6 and 8.

Use of the base-stacking calculations yields a T_m of 56°C for Promega's T7 Primer when magnesium contributions are taken into account (and 47°C when they are not).

It is apparent that all three methods give similar, but different, values for primer T_m. In most cases any one of the formulas will yield an adequate approximation of the actual T_m of the oligo but for best results the optimum annealing temperature will need to be determined empirically using the theoretically calculated T_m as a starting point.

Molecular weight of single stranded DNA

The molecular weight of a specific segment of DNA is equal to the sum of the molecular weights of each of the nucleotides. For a know sequence, this is calculated as the sum of the molecular weights of each nucleotide monophosphate (adjusted for the phosphodiester bond)[c]:

Molecular weight[d] = (329.2 * number of G's) + (313.2 * number of A's) + (304.2 * number of T's) + (289.2 * number of C's)
This molecular weight is adjusted by -78 for an assumed missing 5' phosphate group (PO_3) which is replaced by a single hydrogen and +17 for a 3' hydroxyl . This must be adjusted by +78 if a 5' phosphate is present.

Notes

[a]We have found that most melting temperature calculations do not take into account the effects of magnesium on helix stability. Therefore, most empirical guidelines used to design experiments will not apply when the magnesium effects are included. We have included the option to consider magnesium in the equation if it is desirable but have not included it in the default setting. Including magnesium will generally raise the theoretical melting temperature by about 5–8°C for oligonucleotides in a 1.5mM Mg^{2+} solution (8,9).

[b]The concentrations of the primer and the target sequence will change dramatically during PCR*, but generally this will not make a significant difference to the calculated T_m. A standard 50µl reaction may contain 0.1µg of human genomic DNA as a template and is 0.5µM for each primer. This reaction would be approximately 2 femtomolar (2×10^{-15}M) for each single copy target. At the end of 30 cycles, this same reaction may produce about 0.5µg of a specific 1kb amplimer that gives a final concentration of 15nM. In this case the primer concentration will not change significantly and thus will remain much greater than the target concentration (6,8).

(c)The molecular weight of the each nucleotide used in calculations was decreased by 1 in Sepetember, 2003 to correct an error.

[d]The molecular weights of dNMP's are 18 greater than those shown in this equation because dNMP's have 3'-hydroxyl groups and 5'-hydrogens that are lost during polymerization.

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